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EXPERIMENTAL STUDIES ON THE PREPARATION AND EFFECTS OF ANTITOXINS FOR TUBERCULOSIS.

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The workers in this field have not been idle, though it must be conceded that few definite good results have been attained, and but little progress has as yet rewarded the patient toil expended in attempts to produce a curative or antitoxic serum for tuberculosis.

Simultaneously with the development of tetanus and diphtheria antitoxin—even earlier (1888)—Héricourt and Richet[33] reported favorable results in transferring immunity to tuberculosis from dogs to rabbits by serum injections. Unfortunately, clinical tests of the efficacy of this serum when applied to human tuberculosis did not prove at all encouraging. Nevertheless, the efforts of experimenters to advance existing knowledge of the toxic products of the tubercle bacilli, to produce an artificial immunity against tuberculosis in animals, and to obtain an antitoxic serum for this disease have been steadily carried on, and some light has been thrown on this all-important and complex problem.

One of us (Trudeau[88 89]) has been engaged in experiments on immunity to tuberculosis since 1891. The present paper, however, includes only our studies with serums which were commenced in 1894. While confessing our disappointment at the outcome of most of these experiments, we yet feel warranted in presenting them, because they seem to us to indicate some interesting phases of work in tuberculosis and the care needed to make safe deductions from laboratory experiments.

By analogy the efficacy of any antitoxic serum and the ease with which it is obtainable would seem to be in direct ratio to the degree of toxicity possessed by the poison against which protection is sought. For this reason, to produce and demonstrate antitoxic properties in serums would, *à priori*, be more difficult in a disease so chronic as tuberculosis, on account of the low degree of toxicity of its products. Our attempts to produce serums by our own methods, the antitoxic power of which could be proved experimentally on animals; or to demonstrate antitoxic properties in the serums produced by others, have shown only slight

potency for any of them up to the present time. Tuberculosis does not belong to that class known as the acute infectious diseases which kill by acute toxæmia, but to the group known as the infectious granulomata to which syphilis, actinomycosis, and leprosy also belong, and which destroy life not only by the chronic and long-continued systemic poisoning they produce, but by the pathological changes brought about through the localization and growth of the germs in organs necessary to life.

Koch's[47] assumption seems plausible, that immunity to the toxic products of tuberculosis does not necessarily imply immunity to tuberculosis, and a serum which would neutralize the toxic effects of tuberculin may not prevent the growth of the tubercle bacillus in the tissues and its destructive action on the organs of the body. An efficacious serum for this disease would probably require, therefore, to possess not alone antitoxic, but also germicidal properties; or, at least, the power to excite the organism to germicidal activity. Bactericidal properties have been claimed for certain serums by careful observers, but the specificity of any such action is open to question and the existence of any demonstrable degree of germicidal power in antituberculous serums has not been confirmed generally by other observers.

It is not at all certain that even if bacterial immunity could be produced by any method, the serum of animals possessing this immunity would necessarily be either antitoxic or germicidal. Indeed, one of us (Trudeau[88]) has succeeded in producing a marked degree of immunity in rabbits by preventive inoculation of living cultures of tubercle bacilli attenuated by prolonged cultivation; and yet the serum of these animals which had resisted a subsequent virulent inoculation proved to have but slight if any antitoxic power, and did not seem to influence to an appreciable degree the course of the disease in tuberculous guinea-pigs.

Notwithstanding the meagre results obtained in animal experiments, a good deal of clinical evidence as to the value of serum-treatment has been presented, which, however, is not sufficiently encouraging to be convincing in tuberculosis, a disease which runs without any specific treatment so varied and erratic a course. The clinical evidence has not been considered in this research, and must be judged by itself. These studies have been entirely confined to such experimental proof of the presence of curative and antitoxic properties in serums as could be obtained by laboratory methods.

The nature of the bacterial poisons used in injecting animals with a view to producing curative serums is probably of vital importance to the success of such attempts.

When we began this work the observations of Hammerschlag,[32] Koch,[46] Proskauer and Brieger[71], Hueppe and Scholl,[39] Weyl,[95] Héri-

court and Richét,[34] Crookshank and Herroun,[23] Richét,[76] Bâbes,[4] Zuelzer,[97] Klebs,[45] Hahn,[30] Kühne,[49] Hoffman,[38] Matthes,[63] de Schweinitz,[82] and our own agreed in the main as to the presence of poisonous albuminous substances in cultures of tubercle bacilli, which were products of the germ-growth and had chemical reactions like the albumoses, albuminates, and nucleo-proteids,* and all producing the characteristic physiologic action of tuberculin. It is to be noted that large quantities of the filtered culture fluid are borne by healthy animals without immediate toxic effects, while quite small doses may produce death in tuberculous animals within a few hours.

No material addition to our knowledge of these culture-products has been published to the present time. Behring[9] has recently reported the separation of a more active poison than hitherto oqtained. De Schweinitz and Dorset[83] have prepared small quantities of a necrotizing substance. More recently Hahn[31] obtained from crushed living tubercle bacilli a juice having the properties of a hydrolytic ferment. The tubercle bacilli have been found by de Schweinitz,[81] Koch and Proskauer,[47] and Unna[91] to contain considerable quantities of fat and cellulose, the former having the specific staining reaction. The whole subject evidently requires exhaustive study.

The experiments with dead tubercle bacilli and their extracts by Wyssokowicz,[96] Maffucci,[54 55] Daremberg,[24] Prudden and Hodenpyl,[73] Koch,[46] Straus and Gamalëia,[86] Vissman,[94] Kostenitch,[48] Grancher and Martin,[29] Grancher and Ledoux-Lebard,[28] Freudenreich,[27] Masur and Kockel,[62] Abel,[1] Carriére,[21] Sciolla,[80] and Bâbes and Proca,[7] show their marked locally irritant character, and their power to produce tubercles, aseptic abscesses, cachexia with grave disturbance of the blood-forming functions, and nephritis. In the face of these facts it seemed hardly probable that it would be practicable to use cultures containing tubercle bacilli, living or dead, to create and increase tolerance. At least it appeared unlikely that animals would tolerate doses presumed to be necessary in order to originate antagonistic substances. That the tubercle bacilli substance is necessary to produce immunity was later claimed by Koch in the experiments with tuberculin "R."

We have endeavored to cover many, but not all, aspects of this subject in our experiments, and our methods have varied in some respects from those of other workers in this field whose developments we have followed. Without further discussing the theoretical considerations concerned, we will proceed to the description of our work.

The studies included in Part I. relate to the methods adopted by us in attempts to produce the sought-for immunity in various animals,

* A single phosphorus determination, kindly made for us by Prof. R. H. Chittenden, of the pure proteid obtained from cultures on synthetic media (containing no peptones nor albuminoid) gave a content of 1.52 per cent. This indicated the presence of considerable nucleoproteid produced by the tubercle bacilli.

and the tests of the germicidal and curative properties which might be possessed by such serums. The studies included in Part II. relate mostly to tests in animals of the antitoxic power of serums in tuberculin poisoning.

Thus far we have employed sheep, fowls, asses, and rabbits in attempts to obtain antitoxic serums. Before giving details of the work the following summary will set forth the general methods employed by us. We obtained serums from :

I. Sheep repeatedly inoculated intravenously with filtrate of cultures of tubercle bacilli on thymus bouillon.

II. Fowls that were repeatedly inoculated intraperitoneally with tubercle bacilli of mammalian tuberculosis of increasing virulence, and recovered.

III. Sheep injected subcutaneously with increasing doses of tuberculin.*

IV. Sheep repeatedly inoculated intravenously with living non-virulent cultures of tubercle bacilli.†

V. Ass repeatedly inoculated intravenously with living, non-virulent cultures of tubercle bacilli.

VI. Ass inoculated : (*a*) Subcutaneously with virulent living cultures of tubercle bacilli ; (*b*) intravenously with virulent tuberculous material, and recovered ; (*c*) treated with tuberculin subcutaneously in increasing doses.

VII. Ass injected : (*a*) Subcutaneously with dead cultures of non-virulent tubercle bacilli on thymus bouillon ; (*b*) with precipitated tuberculin from cultures of the same non-virulent tubercle bacilli on proteid-free media ; (*c*) alkaline extracts of the bacilli with dead bacilli, subcutaneously ; (*d*) living non-virulent tubercle bacilli.

VIII. Rabbits : (*a*) Inoculated intravenously with non-virulent tubercle bacilli and recovered ; (*b*) inoculated intraperitoneally with virulent tubercle bacilli and recovered.

With serums from the foregoing we tried to carry out the following plan of tests :

1. Effect of serum on healthy animals.

2. Treatment of tuberculous animals with serums to show influence on course of disease and temperature.

3. Test of germicidal influence *in vivo* and *in vitro*.

4. Test of power to neutralize effect of tuberculin in small and fatal doses.

* The tuberculin used in this work was made from full-grown bouillon cultures of non-virulent tubercle bacilli from human source, evaporated over a water-bath to one-tenth volume and filtered through clay. 0.100 c.c. usually sufficed to kill guinea-pigs six weeks tuberculous.

† The cultures denoted "non-virulent" were from tubercle bacilli of human origin, grown four years in the incubator, and which only occasionally killed guinea-pigs in six months to one year.

5. Test of effect on local reaction and temperature produced by tuberculin in tuberculous animals.

We must acknowledge at the outset that, for various reasons, we were unable to carry out all of these tests with all of our serums, and the work is incomplete to that degree. Methods used in some tests were changed in others because they were thought to be fallacious, particularly in testing for anti-tuberculin. Consequently there is no true basis for comparison of all the serums tried. In addition to the serums prepared by ourselves, we have tried five or six from other sources. In the present uncertain state of serum-therapy for tuberculosis it is undesirable to mention the names of their originators.

Part I.

I. *Sheep injected intravenously with filtrate of cultures of tubercle bacilli on thymus bouillon.*

June 26, 1894. No. 1, treated, weight 64 lbs.; No. 2, control, weight 64 lbs. Both animals are "wethers" and in good condition. Began injections of filtrate of cultures of (non-virulent) tubercle bacilli on calf thymus bouillon into sheep No. 1. Four doses: June 26th, 10 c.c.; June 30th, 20 c.c.; July 3d, 25 c.c.; July 11th, 25 c.c. Temperature three hours after first dose 103.8.°

July 16*th.* Weights: No. 1, 52 lbs. (loss, 12 lbs.; no other effect noted); No. 2, 64 lbs.

August 4*th.* Weights: No. 1, 57 lbs.; No. 2, 67 lbs.

10*th.* Weights: No. 1, 56½ lbs.; No. 2, 67 lbs. No. 1 did not recover weight, and on this account bleeding was postponed.

October 27*th.* No further improvement or change noted. No. 1 bled 500 c.c. by canula. 175 c.c. serum collected. This animal was so much weakened by bleeding that it was killed ten days later. No lesions found at autopsy.

*Effect of No. 1 Sheep-serum on the Course of the Disease and on the Temperature.**

October 27*th.* Took 6 guinea-pigs, average weight 523 grammes; (*a*) treated with serum, 4 guinea-pigs, average weight of 504 grammes; (*b*) controls. Before inoculation (*a*) received five doses of 4–5 c.c. of the serum subcutaneously; total, 24 c.c. each. No effect to be noted on temperature.

November 5*th.* Weights: (*a*) Treated, average 517; (*b*) controls, average, 511. All were inoculated in groin with virulent material from the lung of a rabbit in which tubercle bacilli were scanty. Each of (*a*) was injected with 4 c.c. of serum.

12*th*, 18*th* and 23*d.* (*a*) Injected with 5, 5, and 2 c.c.; total, 12 c.c. Serum pigs lost more weight than the controls. They showed no local irritation.

* Weight and temperature tables are omitted to economize space.

Result. Average time of death: (*a*) 44 days; (*b*) 62 days. Autopsies revealed generalized tuberculosis of all organs. The serum apparently hastened death and caused loss of weight in healthy animals before inoculation.

A preliminary test of the antitoxic power of this serum on pigs ninety-three days tuberculous showed that it did not prevent a temperature reaction after the use of tuberculin, and the outlook was so unpromising that further tests were abandoned. The bleeding of the sheep being postponed so long, the method may be said to have had hardly a fair trial.

II. *Fowls inoculated intraperitoneally with mammalian tubercle bacilli of increasing virulence.*

September 10, 1894. We took 12 fowls (chickens and cocks), two-thirds grown, having an average weight of 1026 gm. The first inoculation was of 1.5 c.c. of an emulsion of non-virulent tubercle bacilli from bouillon culture which had been grown four years in an incubator oven.

October 27*th.* Average weight 1671. Second inoculation: 0.7 c.c. of an emulsion of non-virulent tubercle bacilli from a bouillon culture which had been cultivated two years.

December 20*th.* Average weight 1717. Third inoculation: 1 c.c. of an emulsion of a virulent culture (cultivated four months on serum).

January 21, 1895. Average weight (10 fowls) 1686. Fourth inoculation: 2 c.c. of an emulsion of a virulent culture (growing five months on serum).

February 15*th.* Average weight (10 fowls) 2006. Fifth inoculation: 1.5 c.c. of the juice of crushed lymph nodes from a rabbit; third passage of virulent tuberculosis.

April 27*th.* Average weight (10 fowls) 1894. Sixth inoculation: 1.5 c.c. of the juice of the lungs and omentum; sixth passage of virulent tuberculosis.

May 22*d.* (Not weighed). Seventh inoculation: 1.5 c.c. of the juice of the spleen and omentum; eighth passage of virulent tuberculosis.

July 19*th.* (Not weighed.) Eighth inoculation: 1.5 c.c. of the juice of the omentum; seventh passage of virulent tuberculosis.

August 10*th.* Average weight 1684. Ninth inoculation: 1.5 c.c. of the juice of the omentum; sixth passage of virulent tuberculosis.

September 4*th.* Tenth inoculation: 1.5 c.c. lung; eighth passage of virulent tuberculosis.

During the course of these inoculations only two of the fowls died, and those from injuries received in fighting. The rest were bled to death at various times after the seventh inoculation. Most of them were bled twelve and twenty-six days following the last inoculation. Only small amounts of serum were obtained, and part was preserved by trikresol. In no case was there found the slightest evidence of a past or present tuberculosis on post-mortem examination.

Test of the Germicidal Power of Fowl-serum.

June 15, 1895. We took an emulsion in water of the first culture of tubercle bacilli on serum (virulent). (*a*) Mixed one-half with 6 c.c. serum; no antiseptic added. (*b*) Mixed one-half with 6 c.c. of 0.6 per cent. NaCl solution. Both were allowed to stand six hours at room

temperature in a dark closet. We then inoculated 3 guinea-pigs, average weight 358 gm., with (*a*) 2 c.c. each; and 3 guinea-pigs, average weight 345 gm., with (*b*) 2 c.c. each. All were inoculated subcutaneously in the right groin.

Result: All became tuberculous in the usual way: (*a*) lived 77 days; (*b*) lived 80 days. There was no germicidal influence *in vitro* on the tubercle bacilli.

The effect of this serum on the temperature of seven tuberculous guinea-pigs and rabbits was tried, with the result that doses of 1.5 to 2 c.c. seemed to cause some elevation in six hours. The amount of serum was insufficient for more of such experiments.

Influence of Fowl-serum on the Course of the Disease in Guinea-pigs.

September 20, 1895. We took 5 pigs, average weight 514.2 gm., for treatment; and 5 pigs, average weight 487.6 gm., for controls. All inoculated with virulent tuberculous material from lung of guinea-pig; each receiving 0.25 c.c. in the right groin. We began treatment the same day, using subcutaneous doses of from 1 to 2.5 c.c. every three to ten days, and giving a total quantity of 10 c.c. each. Abscesses formed in a few instances, probably from skin infection.

Result: The treated animals lived 57 days; the control animals 58½ days.

Tests of the temperature of these animals showed no perceptible influence of the serum. The limited quantity of serum available precluded tests with tuberculin. The results accord with those published recently by Auclair,[3] and were especially disappointing because fowls have such high natural immunity to mammalian tuberculosis and are able to dispose of large quantities of human tuberculous material.

III. *Sheep injected subcutaneously with increasing amounts of tuberculin.*

IV. *Sheep inoculated intravenously with living non-virulent tubercle bacilli cultures and injected with tuberculin in increasing doses.*

April 22, 1895. (III.) Sheep (wether), weight 70 lbs. (IV.) Sheep (wether), weight 65 lbs. Sheep (IV.) received 10 c.c. tubercle bacilli from a non-virulent bouillon culture in the saphenous vein. No harmful effects followed.

May 16th, 10 c.c. same. Weights: (III.) 105 lbs.; (IV.) 80 lbs. After this dose (IV.) lost weight and strength steadily during the summer, so that nothing more was attempted until December of the same year.

December 15, 1895. Weights: (III.) 105 lbs.; (IV.) 80 lbs. *Tuberculin Test.* Injected both with 0.200 tuberculin. The temperature of (III.) rose from 102° to 103.5°; (IV.) reacted from 102° to 105°.

Tuberculin Injections. During the next three months both sheep were injected with gradually increasing doses of tuberculin; at first every second day, then less frequently, according to the loss of weight. (III.) received 19 doses; the largest, 50 c.c.; the total quantity, 184 c.c. (IV.) received 23 doses; the largest, 20 c.c.; the total quantity, 64.5 c.c.

Both animals retained their weight until the maximum dose was reached, which for sheep (IV.) was evidently overwhelming, as it lost 15 lbs. during the following three weeks, finally becoming so weak and

cachectic that it was killed. No tuberculous lesions were revealed by autopsy, but the liver and spleen were found much atrophied; there was a calculus in the pelvis of one kidney. The absence of tuberculous lesions and the apparent good health of this sheep up to the time of the tuberculin injections remind one of the effects mentioned later by Maffucci and Vestea[57] as the result of intravenous inoculations of living tubercle bacilli in sheep. The cachexia in one sheep was probably induced by the tuberculin injections. No signs of hæmoglobinuria were noted in these animals; this is mentioned by Niemann[66] as occurring after massive doses of tuberculin in goats, owing to the large amount of glycerin contained in it.

Sheep (III.) withstood the dose of 50 c.c. of tuberculin fairly well, and was bled March 21, 1896, five days later. 1000 c.c. were taken by canula from the external jugular vein. The weight decreased 20 lbs. during the following three weeks. Part of the serum was kept aseptic and the rest was preserved with camphor.

Test of the Germicidal Power of the Serum of Sheep (III.) Tuberculinized.

March 23, 1896. We took mixtures of: (*a*) 6 c.c. of emulsion in 0.6 per cent. NaCl sol. of a washed sputum rich in tubercle bacilli, adding 12 c.c. of a serum without antiseptic. (*b*) 6 c c. of emulsion of sputum as above; 12 c.c. of 0.6 per cent. NaCl solution. A stained drop showed one or two tubercle bacilli in a field. The mixture was placed in a cool, dark closet for six hours.

We then took 3 guinea-pigs, average weight 692, each of which received 6 c.c. of (*a*) subcutaneously, and 3 guinea-pigs, average weight 702, each of which received 6 c.c. of (*b*) subcutaneously.

Result: All became infected in the usual time, no difference in the course of the disease being observed. (They were therefore used for testing tuberculin later.) Autopsies revealed rather chronic tuberculosis in all. No germicidal influence was manifested by the serum.

Effect of the Serum of (III.) on the Course of the Disease in Guinea-pigs.

March 23, 1896. We took 6 pigs, average weight 721 gm., and treated them with serum; 4 pigs, average weight 766, were taken as controls. All were inoculated in the right groin with one oese of washed sputum emulsion (same as in above experiment).* Injections of serum preserved with camphor were begun on the following day. It was warmed and administered intraperitoneally to facilitate absorption. Doses of 2 to 5 c.c. were continued every second to fourth day for fifty days. The treated animals received total amounts varying from 50 to 65 c.c.

Result: By the twentieth day all the treated pigs were much more emaciated than the controls. One died from peritonitis from puncture of the stomach in injecting of serum. Three more treated pigs died in fifty days from tuberculosis, while the controls were still vigorous. The controls were therefore killed on the fifty-first day for comparison, and the lesions were found practically the same as in the treated. The

* Sputum was used for these inoculations, thinking more nearly to approach the infective power of tubercle bacilli for human beings.

serum appeared to act harmfully; at least when given in the peritoneum, though the two remaining treated animals survived 110 to 132 days, showing lesions which were chronic, but notsunusual in character. The effect on temperature was not noted in above experiment.

Test with Tuberculin of Serum (III.) Antitoxic Power.

Eight pigs inoculated with sputum were tested on the nineteenth, thirtieth, and thirty-third days of disease with serum and tuberculin mixed, given subcutaneously. The conditions of the experiments were so unsatisfactory that they deserve only brief mention. So far as could be judged, no favorable influence was observable. In some pigs the serum seemed to cause fever, and since the bleeding of the sheep was undertaken only five days after a large dose, it is conceivable that some of the tuberculin may have still been contained in the serum.

V. *Ass J.; inoculated intravenously with living, non-virulent tubercle bacilli.*

December 13, 1894. Male ass (J.); weight, 445 lbs. Appeared old, but sound. We injected 7 c.c. of an emulsion of tubercle bacilli in 0.6 per cent. sterile NaCl solution into an ear vein. (About one-third went into the subcutaneous tissue, producing induration and a cold abscess.) No effect could be noted on the health of the ass.

January 15, 1895. Weight 460 lbs. We gave successfully 15 c.c. of an emulsion of tubercle bacilli in 0.6 per cent. NaCl solution; the tubercle bacilli were taken from three bouillon tubes. No injurious effect was seen.

March 7th. Weight 480 lbs. We injected 15 c.c. of a similar emulsion of tubercle bacilli taken from 6 tubes, with partial success.

April 22d. Weight, 490 lbs. We attempted to inject 10 c.c. of a strong emulsion of tubercle bacilli in 0.6 per cent. sterile NaCl solution into the external jugular; most of it went into the subcutaneous tissues.

May 16th. Weight, 475 lbs. We gave 12 c.c. of a thick emulsion of tubercle bacilli in 0.6 per cent. sterile NaCl solution into an ear vein.

Result: Died in twelve hours from an embolus in the pulmonary artery. The emulsion was probably too thick and produced a clot. No evidence of tuberculosis of the lungs, liver, or spleen could be found at autopsy, nor on microscopical examination of sections. Enough serum was saved from the heart cavities to test its bactericidal influence.

Test of the Bactericidal Effect of Serum. Ass J. (V.).

May 18, 1895. (*a*) 4 c.c. of the above serum, unfiltered, was mixed with 1 c.c. of the crushed and strained juice from the spleen of a tuberculous guinea-pig that had died in five weeks; (*b*) 2 c.c. 0.6 per cent. NaCl solution was mixed with 0.50 c.c. of the same juice. Both stood five and a half hours in a dark closet. 4 pigs, average weight 315 gm., received 1.25 c.c. each of (*a*). 2 pigs, average weight 293 gm., received 1.25 c.c. each of (*b*) in the right thigh.

Result: All became uniformly tuberculous as usual, indicating that the serum had no effect on the bacillus, though the experiment is obviously inconclusive.

VI. *An ass was inoculated subcutaneously and intravenously with virulent cultures of tubercle bacilli and virulent tuberculous material from animals; it was then injected with tuberculin in increasing doses.*

February 25, 1895. *Subcutaneous inoculations.* Female ass (R.); weight, 450 lbs.; full-grown, but young; in fine condition. The normal rectal temperature varies from 98.4° to 99.5°. The first inoculation was of 12 c.c. of an emulsion of the liver and omentum of a rabbit that had died in fifty-one days of acute tuberculosis (fourth passage through rabbits). There were few tubercle bacilli in the emulsion; a cheesy abscess resulted, which was opened and healed.

April 6th. Weight, 455 lbs. Second inoculation: 12 c.c. of an emulsion of the liver and omentum of a rabbit that had died forty days (fifth passage); tubercle bacilli were fairly numerous. An abscess followed.

May 22d. Weight 430 lbs. Third inoculation: 4 c.c. of an emulsion of the liver and omentum of a rabbit that had died in twenty-two days (seventh passage); tubercle bacilli were few. Injections were made in three or four places; no abscesses followed, but there was permanent induration.

June 9th. Fourth inoculation: 4 c.c. of an emulsion of the liver and omentum of a rabbit that had died in forty-nine days (seventh passage); tubercle bacilli fairly numerous. An abscess resulted; otherwise its health remained good. It was turned out to pasture for the summer.

August 9th. Intravenous inoculations. First inoculation: 5 c.c. of an emulsion of the lymph nodes and omentum of a rabbit three months tuberculous (seventh passage); it was injected into an ear vein; tubercle bacilli were scanty in the emulsion. No disturbance of health that was perceptible followed.

24th. Second inoculation: 2 c.c. of an emulsion from the lymph nodes and omentum of a rabbit three months tuberculous (eighth passage); tubercle bacilli were numerous. Following this the ass soon became daily thinner and weaker, and breathed somewhat quickly.

September 1st. Losing weight. Evening temperature 100.2°.

13th. Injected with 0.030 tuberculin; reaction to 104°.

November 19th. Gaining weight; evening temperature 100°.

20th. Tuberculin injections. Begun with 0.030, and gradually increased every two to ten days for five months. Thirty doses were given, aggregating 173.5 c.c. The last dose was 33 c.c. Her weight gradually increased from 350 to 420 lbs., and the reactions became less. The last dose caused a temperature of 101°, but no depression. Little disturbance was noted from the tuberculin after the first four or five doses.

May 4, 1896. First bleeding: Ten days after the largest dose, 1500 c.c. of blood was taken from the jugular vein by canula. Some of the serum obtained was preserved aseptically in tubes, while the rest was kept with lumps of camphor added. No depression followed the bleeding, so the animal was turned out to pasture for the summer. During the summer of 1896 ass R. was quite thin, weighing only 345 lbs., but she had regained her weight in the autumn—to 400 lbs. on October 15th.

November 2d. Tuberculin injections were resumed, beginning with a dose of 0.500 c.c., which was followed by a rise of temperature to 102°.

The doses were rapidly increased, causing about the same reaction, until December 29th, nearly two months. The last dose was 10 c.c.; the aggregate 46.5 c.c. An interval of thirty days was then given before bleeding, during which time the ass gained 20 lbs. in weight.

January 27, 1897. Second bleeding: 1800 c.c. was taken from the left jugular.

Test of the Germicidal Power of the Serum of Ass R. (VI.).

First serum: The same method was used as in the fowl and sheep serums except that virulent cultures were employed for infective material.

May 6, 1897. (*a*) 12 c.c. of serum (without antiseptic) was mixed with 6 c.c. of an emulsion of tubercle bacilli from a first culture on serum. (*b*) 12 c.c. of a 0.6 per cent. solution of NaCl was mixed with 6 c.c. of tubercle bacilli emulsion as above. Both were allowed to stand twelve and one-half hours in a dark closet.

We took 3 pigs, average weight 517 gm., and inoculated them with 3 c.c. each of (*a*); and 3 pigs, average weight 512 gm., which we inoculated with 3 c.c. each of (*b*).

Result: After twenty-six days the evidence of uniform disease was so palpable in all that they were killed by tuberculin. All were found to have lesions of generalized tuberculosis.

Second serum: This serum was not tried on animals, but was added to bouillon cultures without heat to test its inhibitive power on the growth of tubercle bacilli *in vitro*.

January 31, 1897. We took 4 flasks containing 50 c.c. each, of bouillon + 10 c.c. or 5 c.c. of serum (R. second); 5 flasks containing 25 c.c. each of bouillon + 2.5, 1.25, 0.63, 0.50, and 0.25 c.c. of (R. second), 4 flasks containing 25 c.c. each, of control bouillon + 10, 1.25, 0.50, and 0.25 c.c. of hydrocele fluid, and 1 flask containing 25 c.c. of control bouillon only.

All were planted with non-virulent tubercle bacilli, surface growth. Only one flask became contaminated. No retardation of growth was seen unless the serum was present in large proportion. In the latter case the alkalinity was so increased by the serum that from comparison with the hydrocele fluid cultures and from former observation we may attribute the lessened growth to the wide differences in this respect.

Result: There was no reason to suspect any antibacillary effect of a specific nature from the above experiment.

Effect of the First Serum of Ass R. (VI.) on the Course of the Disease in Guinea-pigs.

May 4, 1896. 10 pigs were inoculated in the right groin with a virulent culture of tubercle bacilli, the first culture on serum. 6 pigs, average weight 500 gm., were treated with serum. 4 pigs, average weight 520 gm., as controls (*a*). 2 pigs, average weight 515 gm., as controls (*b*); which were not inoculated, but were treated. 1 pig, weight 541 gm., as control (*c*); which was neither inoculated nor treated, but was kept in the same cage for comparison.

Injections of serum into the peritoneal cavity were begun on the fourth day, and were continued every second or third day for fourteen doses, which ranged from 1 c.c. to 6 c.c. The average total amount for each pig was 45.7 c.c.

Result: The treated died on an average of 45.5 days after inoculation. Controls (*a*) died on an average of 42.5 days after inoculation; controls (*b*) died on an average 50 days after inoculation; control (*c*) gained 33 grammes weight.

No effect could be observed either on the appearance of the treated animals or on their temperature and lesions as compared with the controls. The controls (*b*), which were to show the effect of the serum on healthy animals, were inoculated by mistake May 20th. They serve, however, to show that the serum failed to prevent or modify the disease when given prior to inoculation. All died with generalized tuberculosis. The prolongation of life was not sufficient to be significant.

Possibly a more favorable result would have been obtained with this serum had it been used subcutaneously in smaller doses. The quantity at our disposal was too small to carry out a second test and to try its effect on tuberculous eyes of rabbits. The second serum from ass R. was thus tried, as will be seen hereafter. (The experiments on the antituberculin or antitoxic power of first serum, ass R., will be given in Part II.)

Effect of the Second Serum of Ass R. (VI.) on the Course of the Disease.

February 12, 1897. 15 pigs were inoculated in the right groin with a culture of tubercle bacilli of moderate virulence. 5 pigs, average weight 634 gm., were treated with the second serum. 5 pigs, average weight 602 gm., controls.* (*a*) 1 pig, weight 580 gm., control (*b*), was not inoculated, but was treated. 1 pig, weight 455 gm. control (*c*), neither inoculated nor treated.

Injections of serum were begun next day under the skin of the abdomen; doses of from 0.050 to 1 c.c. at intervals of one to three days were given until May 4th—nearly three months. No marked induration of skin nor abscesses resulted. The total for each pig was 17.4 c.c.

Result: 4 treated pigs died after an average of 95.2 days; 1 treated pig survived four months, and was then killed. 2 controls (*a*) died after an average of 75.5 days; 3 controls (*a*) survived four months and were then killed 1 control (*b*) lost 10 grammes in four months. 1 control (*c*) gained 15 grammes in four months.

No influence was manifest on the animals, nor on their temperatures taken before and after doses of 1 c.c., and compared with controls. Nothing unusual was noted in the lesions. All had chronic generalized tuberculosis.

This experiment was in direct contrast to that with the first serum in the less virulent inoculation and with smaller doses of serum given under the skin instead of into the peritoneal cavity. The serums seemed to have no favorable influence either way.

VII. *Ass injected subcutaneously: (1) with dead non-virulent cultures of tubercle bacilli on thymus bouillon; (2) with precipitated tuberculin made from cultures of non-virulent tubercle bacilli on proteid-free media; (3) with alkaline extract of tubercle bacilli mixed with dead tubercle bacilli; (4) with living non-virulent tubercle bacilli.*

January 4, 1895. Female ass (H.), weight 475 lbs., full-grown, rather "phlegmatic;" in good condition and apparently sound; normal rectal temperature—97° to 98°.

* The other five pigs were treated with Serum VII. See page 15.

1. *Injections of non-virulent cultures of tubercle bacilli on thymus bouillon which had been killed by trikresol.* Began injections of emulsion of tubercle bacilli in thymus bouillon culture with 0.2 per cent. trikresol, on alternate sides of neck. January 4, 1895, 1 c.c.; 11th, 1.5 c.c.; February 8th, 5 c.c.; March 4th, 8 c.c.; April 8th, 10 c.c.

No rise of temperature followed these injections, and only indurated spots remained; no abscesses resulted. The animal was not visibly affected, except that she lost 15 pounds, which was regained by April 17th, when she was turned out to pasture for the summer. Nothing further was done until December 11th, when a dose of 0.500 tuberculin was injected without an ensuing reaction.

December 12th. 2. *Injections of precipitated tuberculin.* We began injections of toxin obtained from cultures of non-virulent tubercle bacilli on liquid media containing asparagin, mannit, or ammonium carbonate in mixtures with salts as in the formulas of de Schweinitz[84] and Proskauer and Beck.[70]

The toxin was prepared as follows: To the clay-filtered culture fluid 2 per cent. acetic acid was added, followed by ammonium sulphate to saturation. Nearly all the proteid matter arising from the fully grown cultures was thus precipitated. It was then collected, redissolved in water, reprecipitated by alcohol, filtered, washed with alcohol and ether, dried, and weighed. Solutions of the solid substance, 0.005 in 1 c.c., were made in weak sodium carbonate. 0.5 c.c. of this preparation was not fatal to tuberculous guinea-pigs, though producing high temperature and local irritation at the site of injection.

This pure tuberculin or toxin was injected every second day in increasing doses up to 7 c.c. until January 30, 1896. The total quantity was 26.85 c.c. Abscesses then formed at site of injection, and occasionally thereafter from the following treatment:

3. *Injection of alkaline extract and dead tubercle bacilli.* The toxin was next prepared by adding 1 per cent. NaOH to the cultures before filtration; warming to 40° C.; filtering through cotton; precipitating by 2 per cent. acetic acid, which gave an abundant flocculent substance: filtering again; washing out the acid with water by decantation; re-solution in weak alkali. Naturally this fluid contained many dead and disintegrated tubercle bacilli, as the filtration was carried through cotton and paper. We found it impracticable to filter through clay, since most of the dissolved poison was left on the filter.

From February until June, 1896, fifteen injections were given subcutaneously, in increasing doses, of these solutions well diluted and freshly made. The largest amount represented 3500 c.c. of cultures. In the aggregate, 17,500 c.c. of cultures grown in 100 to 150 c.c. flasks were used. Considerable induration persisted after these injections, and on three occasions they were followed by septic abscesses which necessitated an interruption of the treatment. The solutions not being sterilized, and the dissolved poison and dead bacilli both being such strong irritants, we despaired of further increasing the doses. Throughout the time of treatment the ass had but little disturbance of health except at the time of the abscesses, when she did not eat well. The abscesses were opened as soon as formed, and healed promptly. By June 1st she had lost fifteen pounds, but regained it by the 15th inst.

June 24, 1896. First bleeding: Nine days after the last dose of toxin and tubercle bacilli, 1000 c.c. of blood was drawn from the right

external jugular vein; 400 c.c. of serum was obtained aseptically. All the abscesses had healed at this time except two or three small pustules. The animal was then put into the pasture. Although this had been an unpromising experiment, owing to the abscesses, we decided to try the serum, omitting the germicidal test on animals.

Effect of First Serum, Ass H. (VII.), on Course of Disease in Guinea-pigs.

June 26, 1896. 10 pigs inoculated in right groin with tuberculous material from a guinea-pig; virulent infection. 6 pigs, average weight 710.8, treated with serum; 4 pigs, average weight 713.7, (*a*) controls; 2 pigs, average weight 780, (*b*) controls, not inoculated, but treated with serum.

The serum (with camphor added to insure preservation) injections into peritoneum were begun on next day and given every second day for a month with doses of 1 to 7 c.c.; total quantity, 41.5 c.c. each.

Result: Average time of death: treated 47 days; (*a*) controls 49.7 days. Controls (*b*) lost weight and were inoculated later, but developed the disease in usual way. The temperatures of all were taken on the twenty-second day of disease and after the dose of 7 c.c. serum, without showing any influence from serum. The antituberculin tests will be found with the other serums in Part II.

4. *Ass inoculated subcutaneously with non-virulent living cultures.*

November 7, 1896. Took ass H. (same as was used in VII.) after being in pasture all summer. Weight 490 lbs. Indurations from former treatment remained, but were smaller.

The first inoculation was of 25 c.c. of culture fluid with tubercle bacilli rubbed up in mortar, weighing in moist condition 0.0221—from an actively-growing culture of non-virulent tubercle bacilli on acid bouillon. The injection was into the right shoulder. The temperature six hours later was 99°.

November 20th. Second inoculation: 25 c.c. of a culture containing 0.1353 tubercle bacilli into left shoulder. Several small aseptic abscesses from the first inoculation were to be seen.

December 7th. Third inoculation: 25 c.c. (the tubercle bacilli were not weighed, but more were used); the injection was made into the right groin; there was no abscess from last dose.

19th. Fourth inoculation: 40 c.c. into the left groin; no abscess resulted.

January 6, 1897. Fifth inoculation: 40 c.c. into the right shoulder; temperature 98.5; no abscess followed.

Much induration of the skin remained after all the inoculations, but little disturbance of health was noticeable. Weight 500 lbs.

February 6, 1897. Second bleeding: Thirty days after the last dose, weight 510 lbs.; 2000 c.c. blood was taken from the right jugular vein; 1000 c.c. serum was collected aseptically.

Germicidal Power of the Serum, Ass H., in Culture.

February 13, 1897. Second serum: We took 2 flasks each, containing 50 c.c. bouillon + 10 c.c., 5 c.c., or 2.5 c.c. serum; 2 flasks each, containing 50 c.c. bouillon + 2 or 1.25 c.c. $\frac{1}{10}$ normal serum, and 2 control flasks each, containing 50 c.c. bouillon + 2.5 c.c. or 1 c.c. of NaOH $\frac{N}{10}$ solution.

The serum was added aseptically, no heat nor antiseptic being used. The NaOH was added to the controls because the serum increased the alkalinity of the bouillon. All were planted with non-virulent tubercle bacilli from a bouillon-serum culture.

Result: March 20th, thirty-five days after, all had grown luxuriantly; there were no contaminations; no inhibitive influence of the serum was to be seen.

Effect of the Second Serum of Ass H. on the Course of the Disease in Guinea-pigs.

February 12, 1897. 15 pigs were inoculated in the right groin with the culture of tubercle bacilli of moderate virulence. 5* pigs, average weight 620 gm., were treated with serum subcutaneously. 5† pigs, average weight 602 gm., (*a*) controls, were not treated. 1 pig, weight 575 gm., (*b*) control, was not inoculated, but was treated. 1 pig, weight 455 gm., (*c*) control, was neither inoculated nor treated.

The doses and intervals were the same as in the experiment with the serum of ass R., the two serums being compared in this way. (See page 12.)

Result: 4 treated pigs lived on an average 106.7 days; 1 survived 4 months; 2 controls lived on an average 75.5 days; 3 survived 4 months. No influence could be ascribed to the serum so far as the lesions or the course of the disease were concerned.

Effect of the Second Serum of Asses R. and H. on the Course of Eye-tuberculosis in Rabbits.

January 30, 1897. 12 rabbits were inoculated in the anterior chamber of the left eye with one drop of weak emulsion of a pure culture of tubercle bacilli of moderate virulence. (R.) 4 rabbits, average weight 2028 gm., were treated with serum from Ass R. subcutaneously. (H.) 4 rabbits, average weight 1741 gm., were treated with serum from Ass H. subcutaneously. (C.) 4 rabbits, average weight 1705 gm., were used as controls.

February 12th. Twelfth day. We began treatment with doses of from 2 to 6.5 c.c. of the serums every second or third day, under the skin of the abdomen; treated until March 23d—forty days; the total amount given each was 66 c.c.

Result: The serum produced induration, but no abscesses nor disturbance of health. There was no change in the appearance of the eyes in the treated which was not seen in the control rabbits; no difference in temperature was noted when taken six hours after the first dose of serum; the disease in the eyes ran the usual course.

VIII. *Rabbits:* 1. *Inoculated intravenously with non-virulent cultures of tubercle bacilli; recovered.* 2. *Inoculated in peritoneal cavity with virulent tuberculous material; disease arrested or recovered.*

March 31, 1896. We took 3 rabbits, weights 2210, 2210, 2085 gm. Each received 1 c.c. of an emulsion of a pure culture of non-virulent tubercle bacilli in water into the aural vein. After temporarily losing weight they appeared completely well six months later.

* Same lot as "second serum, ass R.;" page 12.

† Same controls as in "second serum, ass R.;" page 12.

October 3d. All were inoculated intraperitoneally with an emulsion from a caseous lung of a monkey. The three control rabbits all died in two months. All the above animals survived and were apparently recovered by January 2, 1897, when they were bled to obtain serum. Some chronic lesions were found in one animal, but were not progressing.

Effect of the Serum of Rabbit (VIII.) on the Course of the Disease.

January 5, 1897. We took 5 pigs, average weight 555.2 gm., for treatment; and 5 pigs, average weight 553.4 gm., for controls. All were inoculated in the right groin with a pure culture of tubercle bacilli of weak virulence.

7th. We gave subcutaneous doses of 0.050 to 0.850 c.c. of the rabbit serum every second to third day, until February 21st—forty-five days. Total quantity, 9.65 c.c. each. Induration of skin was produced, but there were no abscesses; no other effect was perceived.

Result: 4 pigs treated died after an average of 92.5 days; 1 survived 4 months and was then killed. 3 control pigs died after an average of 77.3 days; 2 survived 4 months and were then killed. No difference in the lesions was to be seen on post-mortem examination, but the serum pigs that died outlived the controls that died. In a single tuberculous pig no effect on the temperature was produced by a dose of 1.70 c.c. The limited quantity of this serum precluded further investigation.

In addition to the serums prepared by ourselves, we made a preliminary test of some serum said to be from a horse treated with toxins obtained from virulent bacilli. We injected three tuberculous pigs every other day for two months without apparent effect in prolonging life, and hence were unable to confirm the author's statements regarding the serum.

We must state with reference to all the foregoing experiments in treating pigs with serums that they were only preliminary, and the results with so few animals are not viewed by us as conclusive.

Part II.

Tests of the Antitoxic Power of Serums in Tuberculin Poisoning.

In these experiments we have considered:

1. Fatal doses of tuberculin* in sound animals.
2. Fatal doses in tuberculous animals.
3. Small doses in tuberculous animals to show the effect: (*a*) on the temperature; (*b*) on the local reaction.

Many difficulties present themselves in any attempt to demonstrate a specific antitoxin in tuberculosis by methods analogous to those used so satisfactorily in diphtheria. The principal of these relate to the

* Under the term "tuberculin" we here include the various extracts of tubercle bacilli, but usually mean the original Koch fluid.

difficulty of obtaining a highly poisonous product in small bulk unless tuberculous instead of sound animals are employed for the tests.

Objections can easily be made to the use of tuberculin and diseased animals for testing serums. In the first place, tuberculin when prepared by boiling heat may not represent the unaltered toxins of tuberculosis, though containing more toxic extractive substances from the bacilli than when made by evaporation of cultures at low temperatures. Yet we cannot safely say that tuberculin is a wholly altered product until we have further knowledge of its chemical nature. We know, indeed, that boiling heat not too prolonged does not destroy its most prominent physiologic action on tuberculous animals.

The propriety of using tuberculin in serum tests principally depends upon the view one may take of the character of the peculiar reaction. That question may need further elucidation, but it seems to us that the best explanation we have from observations up to date supposes tuberculin to be a *partly specific irritant, both to tuberculous foci and to the susceptible organism in general.* The local and general reaction is caused partly by the poison contained in the tuberculin, which irritates the sensitized cells composing the tubercle, and partly by those toxins set free by the hyperæmia or the enzyme (?) action directly or indirectly produced by the dose of tuberculin (see Bâbes and Proca[7]). Whether the toxins liberated are the same, chemically, as those obtained from artificial cultures of tubercle bacilli is not easy to determine; but that some are set free seems obvious from the profound general disturbance brought about by a minute dose of tuberculin. According to this theory, the poisons are stored up in the tubercles, and in part at least derived from the dead or weakened bacilli, as shown by Bâbes and Proca[7] in experiments with dead tubercle bacilli. Crookshank and Herroun[23] and Matthes[63 64] have extracted albuminous poisons out of caseous material from tuberculous lesions which had the properties of tuberculin. Kahler[43] and Lenoir[51] found albumoses in the urine of patients being treated with tuberculin, which produced tuberculin reactions and were present in the urine in excess of the amount injected as tuberculin. Thus we have some evidence of the discharge of accumulated pyrogenic substances; and that the tuberculin injected, in the minute dose necessary to cause the reaction, may not itself play the prominent part as a poison, but rather as the excitant to the discharge of other specific poisons.

Therefore, but for the uncontrollable amounts of hoarded toxin exploded, as it were, coupled with the local congestions, there would be no objection to proving the activity of serums in this way. Given the serum of a tuberculous animal which had been treated with tuberculin and had tolerated the reactions well, we might hope, if an anti-body had been in the serum, that it would be effective against the specific poisons developed in the animal body by tuberculosis, though not necessarily

against tuberculin as obtained from cultures. By analogy, such an antitoxin ought also to manifest its activity on other tuberculous animals injected with a dose of tuberculin just large enough barely to produce a reaction (and at the same time with a dose of such serum sufficient surely to neutralize it). Should it be weak in activity, it would still seem possible to obtain some effect in that way. Furthermore, it might reasonably be expected that the fatal effects of doses of tuberculin just sufficient to cause death in tuberculous animals with uniform lesions might be inhibited by the administration of an efficient serum; or, at least, that the lives of the animals might be appreciably prolonged. Such tests ought to give qualitative results, though, from the unknown quantities of toxin to be dealt with, they manifestly would not serve as quantitative standards for serums.

On the other hand, healthy animals cannot be suitable for tests until we can obtain sufficiently toxic unaltered products of tubercle bacilli; while if the serums proved to be wholly or chiefly bactericidal, and not antitoxic, they would not reveal that fact in non-tuberculous animals.

As is well known from Koch's original experiments, healthy guinea-pigs tolerate very large doses of tuberculin. This fact renders it necessary to employ such a quantity of ordinary tuberculin to produce death that the effects of the unaltered ingredients of the culture fluid, such as glycerin, albumoses, or peptone, should be remembered, as well as the shock produced by the traumatism of large injections.

We occupied ourselves for some time in attempts to produce a concentrated tuberculin or toxin from cultures; but while it is comparatively easy to produce tuberculin, even from non-virulent cultures, which will usually be fatal in doses of 1 c.c. to 100 grammes of pig (the strength of toxin mentioned by Maragliano as used to test his serum), yet this ratio did not suffice in our experiments to kill pigs weighing above 250 grammes. In addition to the objections mentioned, we found its absorption from under the skin very uncertain.

We then sought by precipitation from cultures on proteid-free media to obtain a pure toxin which would be fatal in small doses. We also attempted to procure a concentrated poison from the bacilli by using weak alkaline extracts, but none of our preparations were quickly fatal to healthy animals in small doses, though producing cachexia and death after some time. After testing various preparations subcutaneously and intraperitoneally, without encouragement, we abandoned the idea of utilizing healthy animals for antitoxin or antituberculin tests. In the course of this work we used fifty guinea-pigs, weighing from 150 to 450 grammes. More recently, fresh extracts from crushed, living tubercle bacilli gave no more uniform fatalities.

Clinical experience with human tuberculosis confirms the fact that

the poisons are of relatively low toxicity—where their immediate effects are considered—as compared with those of diphtheria, tetanus, etc. The same fact suggests itself as a reason for the indifferent results of experiments in sero-therapy with tuberculosis. The effect of tuberculin administered to an animal after the disease becomes established is, nevertheless, so powerful, and the dose so small, that the objections mentioned do not enter.

A surely fatal dose of tuberculin for a tuberculous guinea-pig, though not uniform, is small enough for testing serums; yet there are other factors which are not to be disregarded in such experiments, and which are very difficult to control. The local reaction varies considerably in intensity in the same series of animals. Differences in virulence of the inoculation material used, variations in lesions and in the extent of caseation, the age, and the frequent pregnant state of the animals, suffice to require large numbers of animals in any experimental work on tuberculosis. All these factors we found especially important in testing for anti-tuberculin.

For the reasons mentioned, the fatal dose of tuberculin necessarily varies. Differences in the stage of the disease, the injection of serums mixed with tuberculin, or given separately may produce diverse results owing to different rate of absorption* or other reasons. We also found that non-tuberculous serums, and even physiological salt solution when used under like conditions, gave results that dampen over-enthusiasm in making deductions. Yet in the experiments that follow, in the course of which two hundred and fifty pigs were used, there can be seen indications of a favorable influence or possibly an antitoxic effect from some of the serums on tuberculous guinea-pigs tested with fatal doses of tuberculin.

Details of the Methods Used to Test the Antitoxic Power of Serums.

Guinea-pigs were inoculated in lots of ten to twenty, at different times, with cultures or other tuberculous material of varying virulence. The point of inoculation was usually below the right groin in the subcutaneous tissue.

The amount of tuberculin used to kill them varied from 0.100 to 0.500 grammes, according to the stage and type of disease in the animals; it was made from non-virulent bouillon cultures. The same bottle was used in nearly all the tests. To test the effect on temperatures doses of 0.001 to 0.002 were employed.

The doses of serum ranged from 1 to 12 c.c. Some lots were tested with doses of serum and tuberculin proportioned to the weight of the pigs.

* The slow absorption of the infiltration produced by injections under the skin of guinea-pigs of sheep, cow, ass, and rabbit serums was a point specially noted by us in all this work, and has been of late particularly contrasted with the better absorption of horse serum by Uhlenhuth.[90]

Loss of weight and strength were taken as the most practical criteria of the condition of the diseased animals at the time of testing.

The tests were made from twenty-one to fifty-six days after infection.

To avoid false deductions from lack of absorption of tuberculin when mixed with serum, it was given separately in most of the experiments.

Serums were injected at the same time or before the tuberculin, subcutaneously, or, in some instances, intraperitoneally; the tuberculin always under the skin. Temperatures were taken in the rectum at the time of injection and six hours later. Some of the surviving animals were usually killed for comparison after twenty-four hours.

Normal horse serum, diphtheria antitoxin, normal sheep serum, 0.6 per cent. NaCl solution, and hydrocele fluid were used to control antituberculosis serums in comparative tests.

Serum of Ass R, First Bleeding.

March 23, 1896. 16 guinea-pigs of an average weight of 692 grammes were inoculated in the groin with a non-virulent sputum, which kills in from 60 to 100 days.

May 7th. We tested 12 *pigs on the forty-fifth day.* Each received serum subcutaneously in doses of from 1 to 8 c.c. mixed in 7 instances with 0.300 c.c. of tuberculin. (I.) Of the 7 pigs that received serum and tuberculin 5 survived, while 2 died in from 13 to 15 hours. (II.) The 2 pigs that were injected with serum only survived and showed no effect. (III.) 3 pigs, controls, received tuberculin only and died in from 9 to 10 hours. Three days later (II.) received 0.300 c.c. tuberculin and died in from 10 to 12 hours.

Result. The infiltrate was not well absorbed. The lesions were not very uniform, but the result seems favorable to the serum, except that there was an insufficient number of controls. No protection was seen when the serum was given three days before the tuberculin.

May 13, 1896. 4 *pigs were tested on the fifty-first day.* The serum was given intraperitoneally in doses of from 8 to 10 c.c.; the tuberculin subcutaneously in simultaneous doses of 0.300 c.c. 2 pigs that received serum and tuberculin died in 10 to 12 hours; 2 pigs used as controls and receiving tuberculin only, died in 10 hours.

Result. Inconclusive; there was no better absorption of the serum infiltrate.

April 28th. 12 pigs of an average weight of 529 grammes were inoculated in the groin with sputum that was of moderate virulence (though two died in from 28 to 29 days).

May 28th. 10 *pigs were tested on the thirtieth day.* The serum was given subcutaneously in simultaneous doses of from 3 to 12 c.c. Tuberculin in a separate place in divided doses: 12 M., 0.300 c.c.; 6 P.M., 0.200 c.c. Of 7 pigs that received serum and tuberculin, 3 survived, while 4 died in from 9 to 10 hours; control, of 3 pigs receiving tuberculin only, 2 survived; 1 died in 15 hours.

Result. The serum infiltrate was not well absorbed, though the depression from the tuberculin was not marked until after the second dose. The controls had a higher temperature following the first dose. The

lesions were not uniform and the controls were too few. No conclusion was warranted.

July 11, 1896. 20 pigs of an average weight of 496.7 grammes were inoculated intraperitoneally with a small drop of the juice of very virulent tuberculous tissue.

August 1st. We tested 10 *pigs on the twenty-first day.* All were very ill with acute tuberculosis. Serum was given intraperitoneally; tuberculin subcutaneously at the same time to 7 pigs; the dose of the tuberculin was 0.400, of the serum 2 to 8 c.c. 1 survived, while 6 died in from 6 to 22 hours; 3 control pigs that received tuberculin only died in 9 hours.

Result. Autopsies revealed acute miliary tuberculosis very much alike in all. The amount of serum absorption was doubtful. The result seemed slightly favorable to the serum, but the condition of the animals was bad for the test.

Serum of Ass H, First Bleeding.

May 27, 1896. 10 pigs of an average weight of 534 grammes were inoculated in the groin with a non-virulent sputum, which kills in 60 days.

July 17th. They were tested (fifty-first day). Serum was given intraperitoneally in doses of from 2 to 10 c.c.; tuberculin subcutaneously in simultaneous doses of 0.400 c.c. Of 5 pigs that received serum and tuberculin 2 survived and 3 died in from 6 to 9 hours; of 5 control pigs that received tuberculin only, 3 survived, while 2 died in from 6 to 11 hours.

Result. The serum evidently was not absorbed to any extent. The lesions were not uniform, though the controls were of sufficient number to neutralize that objection. Evidently no conclusion was warranted, as too many controls survived.

July 11, 1896. 10 pigs from a lot of 20 were inoculated intraperitoneally with very virulent material (see previous experiment, July 11th); 2 pigs that died were not used; all became very ill.

August 1st. 8 pigs were tested (twenty-first day). Serum was given intraperitoneally in doses of from 2 to 8 c.c.; tuberculin subcutaneously in simultaneous doses of 0.400 c.c. Of 4 pigs that received both serum and tuberculin 1 survived and 3 died in from 5 to 10 hours; the 4 controls that received tuberculin only, died in from 6 to 10 hours.

Result. The lesions were very uniform and were those of acute miliary tuberculosis. The serum was probably unabsorbed, and the condition of the animals was bad for the test. No conclusions were warranted.

Comparisons Between Various Serums, etc.

The following experiments were made to compare the effects of various serums when given with and without tuberculin. In explanation of the serums tried which were other than our own products, it may be stated that:

"Cow D." Was obtained from an animal that had recovered from pulmonary tuberculosis and had been injected with large quantities of tuberculin.

"Horse I." Was said to have been treated with tuberculous "toxins" from virulent cultures without bacilli.

"Horse II." Had been inoculated subcutaneously with non-virulent cultures.

"Horse III." Was said to have been treated with "toxins" from virulent bacilli freed from the culture fluid.

"Ass M." Was a tuberculinized healthy ass.

In addition to the anti-tuberculous serums we used normal horse serum, diphtheria antitoxin from the New York City Board of Health, sheep serum from a healthy sheep, and a 0.6 per cent. NaCl solution.

July 28, 1896. *To test the serum from cow D*, 10 pigs of an average weight of 714 grammes were inoculated in the groin with virulent tissue juice from a tuberculous pig; 5 died before the test in from 40 to 50 days.

September 28th. We tested 5 pigs on the sixty-second day. Serum was given subcutaneously, mixed with tuberculin, to 3 pigs, the serum in doses of 5, 5, and 8 c.c., the tuberculin in a dose of + 0.400 c.c.; all died in from 4 to 7 hours; 2 control pigs received 0.400 c.c. of tuberculin and both died in from 3 to 5 hours.

Result. All the serum was absorbed. The lesions were uniformly advanced caseous tuberculosis. No conclusion could be drawn, as the dose of tuberculin was probably excessive.

August 24, 1896. *To test the serums from cow D and horse I*, 10 pigs of an average weight of 714 grammes were inoculated in the groin with virulent tissue juice from a tuberculous pig; 2 died on the 43d day.

October 7th. We tested 8 pigs on the forty-fourth day. Serum was given subcutaneously, mixed with tuberculin; 3 pigs receiving the serum from cow D in doses of 2.8, 2.6, and 2.7 c.c. + 0.300 tuberculin died in from 6 to 8 hours; 2 pigs that received the serum from horse I in doses of 2.36 to 2.64 c.c. + 0.300 tuberculin died in 8 hours. Of 3 controls that received 0.300 c.c. tuberculin only, 1 survived and 2 died in 8 hours.

Result. The serum infiltrate was not well absorbed, and the dose of tuberculin was too large. No effects favorable to the serums could be seen.

October 12, 1896. *We tested the serums of cow D and ass H, first bleeding.* 10 pigs of an average weight of 657.4 grammes were inoculated in the groin with a pure culture of virulent tubercle bacilli that killed in from 40 to 50 days.

They were tested on the thirty-second day. Serum was given subcutaneously to guinea-pigs in dose of 0.005 to 1 gramme of pig, diluted with equal quantities of a 0.6 per cent. NaCl solution. Tuberculin in a dose of 0.0006 to 1 gramme of pig, diluted, was given subcutaneously 30 minutes later, on the opposite side of the abdomen; 5 pigs received the serum of cow D in doses of from 2.5 to 2.75 c.c. and tuberculin in amounts of from 0.250 to 0.450; 1 survived, 4 died in from 10 to 14 hours; 2 pigs received the serum of ass H in doses of 2.5 and 2.75 c.c. and tuberculin in doses of 0.250 and 0.450; both died in from 9 to 10 hours; 3 controls received tuberculin only in doses of from 0.250 to 0.450 and died in from 9 to 13 hours.

Result. The serum was incompletely absorbed, except in the pig that survived. The lesions were very uniform and showed generalized tuber-

culosis with beginning caseation. Very little, if any, protective influence of the serum could be seen.

August 23, 1897. *Serum of horse II.* 5 pigs of an average weight of 508 grammes were inoculated in the right groin with a virulent first culture.

September 25th. They were tested on the thirty-third day. 3 pigs received serum subcutaneously in a dose of 0.005 to 1 gramme of pig and tuberculin in a dose of 0.200; 1 survived, 2 died in 12 hours; 2 pigs received tuberculin only in the same dose and died in 12 hours.

Result. Slightly favorable to serum protection in one pig.

The following experiment is so instructive that we give the full details in tabular form. The apparently marked protective influence exerted by both the serums and the physiological salt solution illustrates the fallacies in deductions of supposed specific effects of the serum as determined by such tests.

November 23, 1896. *We tested the serums of horse II, rabbit VIII, and ass R in a 0.6 per cent. NaCl solution.* 19 pigs of an average weight of 517 grammes were inoculated with a pure culture of tubercle bacilli of moderate virulence that killed in from 50 to 60 days.

We tested 13 *pigs on the forty-second day* with serum and salt solution, in a dose of 0.006 to 1 gramme of pig, diluted with an equal quantity of water; it was given subcutaneously in the right side, and tuberculin in a dose of 0.0002 to 1 gramme of pig, diluted with water, was given subcutaneously, 45 minutes later, in the left side. 5 pigs received the serum of horse II in doses of 2.58 c.c. to 3.90 c.c.; 1 died in 15 hours, 4 survived; 2 pigs received the serum of rabbit VIII in doses of 3.48 to 3.51 c.c. and both survived; 6 controls received tuberculin only, and all died in from 7 to 11 hours.

We tested 5 *pigs on the forty-fifth day.* 1 pig that received 2.70 c.c. of the serum of ass R survived; 2 pigs that received 0.6 per cent. NaCl, 3.00 to 3.19 c.c., survived; 2 controls died in 10 hours. See Table I. (A) and (B).

Result. The lesions in this lot of animals were very uniform, and the favorable showing for the serums seemed more than a coincidence. The tuberculin reaction was typical in the organs, the spleens being enormously enlarged and ecchymotic; there was free serum in the cavities; other local congestions were marked. The serum infiltrate seemed to be absorbed but slightly in most animals, even after two days.

The results seen in Tables I. (A) and (B) led us to make comparative tests of various non-specific serums and of salt solution under the same conditions. The number of animals in some cases was too small to make the results of any value, but they are suggestive. Some experiments are conveniently tabulated.

November 13, 1897. *The serum of horse II and normal horse serum "N."* 27 pigs of an average weight of 537 grammes were inoculated in the right groin with a culture of fair virulence; 5 died. The serums and tuberculin were given subcutaneously and in different places 45 minutes apart; the serum in doses of 0.006 to 1 gramme of pig, the tuberculin in doses of 0.200 to 0.500 in pigs Nos. 1 to 15, and in doses

TABLE I (A).—TEST OF 13 PIGS ON FORTY SECOND DAY.

No.	Weights. XI/23,'96	Weights. I/4, '97	Gain or loss.	Serum, kind.	Serum, 12 M. Dose.	Tuberc. 12.45 P.M. Dose.	Temp. 12 M.	Temp. 7 P.M.	Condition. 7 P.M.	Died. Hour.	Died. Hours.	Days survived or killed.	Autopsies.
1	430gm.	430gm	0gm.	Horse II	2.58 c.c.	0.086	102.5°	103°	Lively	.....	.....	Survived 17 days	General caseous tuberculosis.
2	680	645	—35	"	3.87	0.129	103.5	101	Fair	.....	.....	Survived 16 days	General caseous tuberculosis (less).
3	460	465	+ 5	"	2.79	0.093	103	101	"	.....	.....	Killed 2d day	Acute tuberculosis, beginning caseation; some infiltrate at site of injection.
4	660	650	—10	"	3.90	0.130	103	102	"	.....	.....	Survived 14 days	General caseous tuberculosis.
5	465	460	— 5	"	2.76	0.092	103.5	101	"	3 A.M.	15	.....	Acute tuberculosis; ruptured spleen; serum partly unabsorbed.
6	610	580	—30	Rabbit I	3.48	0.116	102.5	101	Weak	.....	.....	Survived 1 day	Acute tuberculosis; serum all absorbed.
7	565	585	+20	"	3.51	0.117	102.8	103	Depressed	.....	.....	Survived 1½ day	" " " " "
8	685	620	—65	Control	0	0.124	103.3	95	Dying	9.30 P.M.	9	.....	" miliary tuberculosis; beginning caseation.
9	535	675	+140 (pregn't)	"	0	0.135	102	97	Weak	.....	9	.....	" " " " " "
10	562	550	—12	"	0	0.115	102	98.5	Depressed	10.30	11	.....	" " " " " "
11	540	540	0	"	0	0.108	101.5	105	Weak	.....	11	.....	" " " " " "
12	490	465	—25	"	0	0.093	103.5	.....	Dying	7.30	7	.....	" " " " " "
13	505	515	+10	"	0	0.092	103.5	101	Weak	9.30	9½	.....	" " " " " "
14	590	.....	.....	.....	.....	.....	.....	.....	.....	Dec. 28, 1896		35th day of disease	

TABLE I (B).—TEST OF 5 PIGS ON FORTY-FIFTH DAY.

No.	Weights.	I/7,'97			12.30 P.M.	1 P.M.	12.30 P.M.	7.30 P.M.					
15	425 gm.	450 gm	+25 g m.	Ass R.I.	2.70 c.c.	0.090	101°	104.5°	Lively	.....	.....	Killed 2d day	General miliary tuberculosis; beginning caseation; serum infiltrate still under skin.
16	425	500	+75	NaCl	3.30	0.100	101.6	102	Weak	.....	.....	" "	" NaCl solution absorbed.
17	390	365	—25	"	3.19	0.073	102.5	106	Lively	.....	.....	" "	" " " " " "
18	485	435	—50	Control	0	0.087	101.5	99.5	Weak	11 P.M.	10	.....	" " " " " "
19	499	525	+26	"	0	0.108	102	101.8	"	"	10	.....	" " " " " "

TABLE II.—TESTED 23 ON FORTY-SECOND DAY.

No	Weight. XI/13,'97	Weight. XII/25,'97	Gain or loss.	Serum, kind.	Serum, 12 M. Dose.	Tuberc. 12.45 P.M. Dose.	Temp. 12 M.	Temp. 6 P.M.	Condition. 12 M.	Condition. 6 P.M.	Died. Hours.	Survived or killed.	Autopsies.
1	662gm.	580gm.	—82gm.	Horse II	3.48 c.c.	0.232c.c.	102°	96°	Good	Fair	15	.....	General tuberculosis; beginning caseation of liver and spleen; some infiltrate from the serum.
2	600	480	—120	"	2.88	0.192	103.5	97	"	"	9	.....	General tuberculosis; beginning caseation, liver, etc.; serum infiltrate unabsorbed.
3	490	400	—90	"	2.40	0.160	100	97.5	Poor	Weak	10	.....	General tuberculosis; more caseation of liver.
4	470	360	—110	N	2.16	0.144	100.6	98.5	Fair	"	10	.....	General tuberculosis; beginning caseation; serum infiltrate unabsorbed.
5	620	565	—55	0	0	0.226	101.5	102	"	Fair	...	28 hours	General tuberculosis; beginning caseation; spleen not large.
6	560	500	—60	0	0	0.200	101.8	102	"	"	...	Surv. 12 dys	Gen. tubere.; slight caseation; spleen not large.
7	490	415	—75	N	2.49	0.166	102.6	96.5	"	"	10	....	General tuberculosis; more caseation; serum infiltrate unabsorbed.
8	425	380	—40	II	2.28	0.152	103	98.5	Poor	"	15	.....	General tuberculosis; slight caseation; serum infiltrate fairly absorbed.
9	405	360	—45	0	0	0.144	101.5	96	Good	Weak	10	....	General tuberculosis; beginning caseation.
10	365	340	—25	II	2.04	0.136	102.8	102	"	Fair	...	Surv. 22 dys	General tuberculosis; slight caseation; marked caseation of the lungs.
11	355	340	—15	0	0	0.136	103.8	104.5	"	"	...	" 45 "	General tuberculosis; marked caseation.
12	385	340	—45	N	2.04	0.136	103.8	103.5	Fair	"	...	" 18 "	Gen. tubere.; marked caseation except spleen.
13	465	430	—35	II	2.58	0.172	102.2	96.5	Good	Weak	10	.....	General tuberculosis; beginning caseation; serum infiltrate unabsorbed.
14	435	360	—75	0	0	0.144	104.4	105	Poor	Fair	...	Surv. 22 dys	Gen. tubere.; more caseation; marked in lungs.
15	350	370	+20	0	0	0.074	102.5	101.8	Good	"	15	.....	General tuberculosis; beginning caseation.
16	555	560	+ 5	N	3.36	0.112	102.2	100	"	Strong	...	Surv. 37 dys	General tuberculosis; massive caseation in lungs; not in liver or spleen.
17	500	510	+10	N	0	0.102	102.2	102	"	"	15	....	General tuberculosis; beginning caseation.
18	705	560	—145	0	0	0	97	.....	Weak	Weak	12	Not treat'd; too weak	General tuberculosis; advanced caseation of liver and spleen.
19	495	430	—65	0	0	0.086	103.6	95	Fair	Fair	10	.....	General beginning caseous tuberculosis.
20	470	430	—40	II	2.58	0.086	102	100	"	"	15	.....	General beginning caseous tuberculosis; serum fairly absorbed.
21	560	525	—35	0	0	0.105	103	100.5	Good	"	18	.....	Ruptured liver; general tuberculosis.
22	395	406	+ 5	II	2.40	0.080	102.5	103	"	"	...	Surv. 5 dys	General tuberculosis; beginning caseation; spleen small.
23	400	340	—60	N	2.04	0.068	102.5	99.8	"	Weak	18	.....	General tuberculosis; slight beginning caseation; serum unabsorbed.

TABLE III.

No.	Weights.			Serum.		Tuberc. 12.45 P.M. Dose.	Temp.		Condition. 7.30	Died or killed.		Autopsies.
	XI/2,'96.	I/7,'97	Gain or loss.	Kind.	12.30 P.M. Dose.		12.30 P.M.	7.30 P.M.		Hours.	Days.	
1	705gm.	610gm.	— 95gm.	NaCl	3.66 c.c.	0.122 c.c.	102.4°	100°	Fair	24	1	General caseous tuberculosis; serum infiltrate not absorbed from skin.
2	710	510	—200	Control	0	0.102	95	.....	Dying	8	.....	General caseous tuberculosis.
3	710	630	— 70	"	0	0.126	102	103.2	Fair	...	VI/2,'97	Chronic caseous tuberculosis (Albino).

TABLE IV.

No.	Weights.			Condition.	Serum.		Tuberc. Dose.	Temp.		Condition. 6 P.M.	Died or killed.		Autopsies.
	XII/30,'96	II/8,'97	Gain or loss.		Kind.	Dose.		12 M.	6 P.M.		Hours.	Days.	
1	655gm.	625gm.	—30gm.	Fair (doe)	Ass R	3.75 c.c.	0.125c.c.	103°	100.4°	Fair	15	.....	General caseous tuberculosis; serum infiltrate not all absorbed.
2	545	585	—60	"	" "	2.90	0.097	102.2	102.6	"	...	K. 10	General caseous tuberculosis.
3	610	545	—65	Poor	Ass H	3.27	0.109	102.4	103.4	"	...	2½	General caseous tuberculosis; serum well absorb'd
4	640	585	—55	Good (doe)	" "	3.50	0.117	102.6	99.2	Weak	10	.....	General caseous tuberculosis; serum infiltrate not well absorbed; more caseation.
5	670	620	— 50	"	Horse I	3.72	0.124	102.4	103.2	Fair	...	K. 9	General caseous tuberculosis; moderate caseation
6	445	385	—60	Poor	" "	2.30	0.077	102.6	99.6	Weak	...	D. 9	General caseous tuberculosis; slight beginning caseation.
7	525	410	—115	Ill	NaCl	2.46	0.082	101.4	103.2	Fair	15	.....	" " " "
8	490	425	—65	Fair (doe)	"	2.55	0.095	103	104	"	...	K. 10	" " " "
9	525	440	—85	Poor	Control	0	0.088	101.2	95	Weak	15	.....	" " " "
10	525	470	—55	Good	"	0	0.094	103.2	101.4	"	9	.....	" " " "
11	620	610	—10	"	"	0	0.122	102.2	104.5	Fair	...	K. 9	" " " "
12	655	.....	.....	.....	.....	.....	.....	.....	.....	.....	Died	I/10,'97	Emaciated; acute peritonitis.
13	480	.....	.....	.....	.....	.....	.....	.....	.....	.....	"	I/11,'97	Emaciated; nearest lymph nodes tuberculous.
14	450	.....	.....	.....	.....	.....	.....	.....	.....	.....	"	I/27,'97	Emaciated; nearest lymph nodes and spleen tuberculous.
15	590	.....	.....	.....	.....	.....	.....	.....	.....	.....	"	II/6,'97	General caseous tuberculosis.

of from 0.100 to 0.500 in pigs Nos. 15 to 23. Of 8 pigs that received the serum of horse II, 2 survived and 6 died in from 9 to 15 hours. 6 pigs received the serum of horse "N," 2 survived, while 4 died in from 10 to 18 hours. (See Table II.)

Result. The lesions were fairly uniform, but the test was too equivocal for any value, and the serum infiltration was not so well absorbed as in Table I.

November 2, 1896. *Hydrocele fluid.* 3 pigs were inoculated with a culture of tubercle bacilli of moderate virulence.

They were tested on the sixty-sixth day. Hydrocele fluid (0.006 to 1 gramme of pig) was injected subcutaneously after diluting it one-half; tuberculin, in a dose of 0.0002 gm. to 1 gramme of pig, was given subcutaneously 15 minutes later. (See Table III.)

Result. No conclusion could fairly be made.

December 30, 1896. *We tested the serums of horse I, ass R 2d, ass H 2d, and a* 0.6 *per cent. NaCl solution.* 16 pigs of an average weight of 558.5 grammes were inoculated in the right groin with a virulent pure culture, that killed in from 30 to 50 days.

They were tested on the fortieth day. The serum of horse I was given in a dose of 0.006 to 1 gramme of pig; the serum of ass R 2d, the serum of ass H 2d, and a 0.6 per cent. of NaCl, 0.006, were given in the same dose; the tuberculin was given in a dose of 0.0002 to 1 gramme of pig, 40 minutes after the serum. The serum was given subcutaneously on the left side; the tuberculin on the right side; both were diluted one-half with water. (See Table IV.)

Result. These animals revealed fairly uniform lesions much like those in Tables I. (A) and (B), and at nearly the same stage in the disease. All had caseous retroperitoneal lymph nodes; enlarged, and beginning caseous, liver and spleen, and miliary tuberculosis in the lungs. The amount of caseation varied, being somewhat less in Nos. 6, 7, 8, and 11 (Table IV.) than in the rest. This latter factor seemed to influence the severity of the poisoning by tuberculin, which is usually more marked the greater the caseation and the age of the disease. Therefore the experiments in Tables III. and IV. have little use for safe deductions.

January 5, 1897. *We tested the serums of cow D, ass R 2d, a normal sheep, and* 0.6 *per cent. NaCl solution.* 10 pigs of an average weight of 483.4 grammes were inoculated with a culture of tubercle bacilli of moderate virulence from the same source as in the experiment of November 23, 1896.

February 16th. We tested them on the forty-second day. The serums, etc., were given subcutaneously, diluted with an equal volume of 0.6 per cent. NaCl, giving 0.004 to 1 gramme of pig; tuberculin, diluted, was given subcutaneously, 45 minutes later, in a dose of 0.0002 to 1 gramme of pig; 2 pigs that received the serum of cow B died in from 12 to 20 hours; of 2 pigs that received the serum of ass R 2d, 1 survived and 1 died in 12 hours; of 2 pigs that received the serum of a sheep (normal), 1 survived and 1 died in 9 hours; 2 pigs received NaCl in 0.6 per cent. solution, and died in from 9 to 10 hours; 2 controls died in from 9 to 12 hours.

Result. The infiltrations from the ass and cow serums were not well absorbed; the others were entirely so. The NaCl seemed completely absorbed when the tuberculin was given. The lesions were very uniform. Possibly some protection was shown by the serums.

TABLE V.

No.	Days inoculated.	Weight.		Tuberc. 12 M.	0.6 per cent. NaCl solution.				Temperature.		Survived or died.	Autopsies.
		II/18,'97	Loss or gain.			Dose. 12 M.	Dose. 3 P.M.	Dose. 6 P.M.	12 M.	5.30 P.M.		
1	50	350gm.	—125gm.	0.350	Control	0	0	0	100°	Dying	6 hours	General tuberculosis; slight caseation; reaction.
2	50	475	— 75	0	"	0	0	0	102	104.4°	K. after 24 hours	General tuberculosis; slight caseation; tuberculin not given.
3	50	555	—115	0.555	NaCl	3.33 c.c.	3.30 c.c.	3.50 c.c.	104.2	100.2	15 hours	General tuberculosis; NaCl nearly all absorbed; reaction apparent.
4	50	565	— 55	0.566	"	3.39	3.29	3.50	103.2	101.2	11 hours	" " " "
5	44	430	— 40	0.430	"	2.50	2.50	2.50	103.2	105	Survived	" " " "
6	44	505	+ 70	0.505	Control	0	0	0	102.2	99.4	10½ hrs.	" " " "

TABLE VI. (A).

No.	Inoculation.	Test day.	Weight.		Serum, etc. January 29th.		Temperature. January 28th.		Temperature. January 29th.		Temp. variation.	Condition.	
			XI/5,'96	I/28,'97	Kind.	Dose.	11.30 A.M.	5.30 P.M.	11.30 A.M.	5.30 P.M.			
1	XI/5,'96	85th	395 gm.	510 gm.	Control	0	102.5°	101.6°	101°	101°	0	Fat	
2	"	"	285	340	Rabbit I	1.70 c.c.	102	102	99.2	102	+2.8°	Thin	
3	"	"	475	525	Ass R 2	2.62	102.5	101.2	102	101.4	+1.6	Fat	
4	"	"	445	540	NaCl	2.70	102	101.6	100.2	101.6	+1.4	"	
5	X/5,'96	116th	480	420	Ass R 2	2.10	102	101.4	101.2	102	+0.8	Poor	Died in 18 hours; was quite ill.
6	"	"	380	500	"	1.25	101.5	100.4	101.2	100.8	—0.4	Good	
7	"	"	.....	545	"	2.77	101	100.3	101	100.4	—0.6	"	
8	Not inoculated.		.....	470	"	2.00	.....	.....	101	101.2	+0.2	"	

January 20, 1897. *We tested the serums of horse II and diphtheria antitoxin* (300 *units*). 10 pigs of an average weight of 529.2 grammes were inoculated in the groin with a culture of tubercle bacilli of moderate virulence (2 died before testing).

February 26th. We tested 8 *pigs on the thirty-seventh day.* The serums were given subcutaneously, diluted with an equal volume of water in a dose of 0.005 to 1 gramme of pig (2 to 4 c.c.); the tuberculin, diluted, was given subcutaneously, 40 minutes later, in a dose of 0.0006 to 1 gramme of pig (0.234 to 0.489 c.c.); 4 pigs that received the serum of horse II died in from 7 to 11 hours; of 2 pigs that received diphtheria antitoxin, 1 survived, while 1 died in 6 hours; 2 controls died in from 8 to 11 hours.

Result. The serums were not perfectly absorbed and the dose of tuberculin was so large that no deduction is warranted.

Note.—These animals had been used on the twenty-second day of the disease with the serum of ass R 1st and small doses of tuberculin (see experiment of January 20, 1897).

February 19, 1897. *We compared the serum of ass R, second bleeding, and a* 0.6 *per cent. NaCl solution.* 16 pigs of an average weight of 437.5 grammes were inoculated in both groins with a culture of tubercle bacilli of weak virulence (all but 3 pigs had gained weight at the end of two months).

April 23*d. We tested all on the sixty-third day.* The serum and the NaCl were given, undiluted, subcutaneously in a dose of 1 to 2 c.c.; tuberculin, diluted, was given $1\frac{1}{4}$ hours later, subcutaneously, in a dose of 0.300 c.c.; 6 pigs that received the serum of ass R, 2d, all died in from 7 to 11 hours; 3 pigs that received NaCl solution, all died in from 7 to 9 hours; of 7 controls, 2 survived and 5 died in from 9 to 12 hours.

Result. The serum infiltration was not completely absorbed; the NaCl solution was entirely so. The lesions were very uniform and showed chronic fibro-caseous lymph nodes; very large, slightly caseous spleen; beginning caseation in the liver; miliary tuberculosis of the lungs. The dose of tuberculin was rather larger than was necessary for most of the animals; no influence could be seen.

Serum of ass M. 3 pigs were taken 55 days after infection (*a*); 2 pigs, $6\frac{1}{2}$ months after infection (*b*); tuberculin was given 40 minutes later than the serum. (*a*) 2 pigs received the serum of ass M in a dose of 1 and 2 c.c. subcutaneously, and tuberculin, 0.100 c.c.; 1 survived and 1 died in 10 hours; 1 control-pig injected subcutaneously with tuberculin, 0.100 c.c., died in 12 hours; (*b*) 1 pig received of the serum of ass M 2.5 c.c., and of tuberculin, 0.100 c.c.; it died in 10 hours; 1 control-pig received tuberculin, 0.100 c.c., and died in 12 hours. (See Table V.)

Result. There was no certain protection.

A 0.6 *per cent. NaCl solution in repeated doses, with large doses of tuberculin.* 6 pigs of an average weight of 480 grammes received 4 inoculations on December 30, 1896, and 2 inoculations on January 5, 1897, of a pure culture of tubercle bacilli of moderate virulence; NaCl solution was given in a dose of 0.006 to 1 gramme of pig, and tuberculin in a dose of 0.001 to 1 gramme of pig. (Table V)

Result. The excessive doses of tuberculin and the prolongation of life in the animals that received the NaCl solution suggest that the

influence of the latter may be due to a supporting effect on the failing hearts of the poisoned animals by adding bulk to the blood or diluting and promoting the excretion of the poison.

May 27, 1897. 0.6 per cent. NaCl *solution in a single dose.* 15 pigs of an average weight of 676.7 grammes were inoculated in the groin with a first culture of virulent tubercle bacilli; 4 died in from 5 to 34 days.

July 2, 1897. *We tested* 11 *on the thirty-sixth day.* The NaCl solution was given subcutaneously to six in a dose of 0.010 to 1 gramme of pig; the tuberculin, subcutaneously, in a dose of 0.150 to all, 15 minutes later; of the 6 pigs that received NaCl solution in doses of 4.75 to 7.30 c.c., 2 survived and 4 died in from 6 to 12 hours; of 5 controls that received tuberculin only, 3 survived and 2 died in from 6 to 12 hours.

Result. No protective influence of the NaCl can be seen in this experiment, though it seems apparent in some others. The lesions showed virulent infection, but not uniform caseation.

Effect of the serums on the temperature of tuberculous guinea-pigs (without tuberculin). Serum of ass R, second; serum of rabbit VIII; and solution of the NaCl, 0.6 per cent. Miscellaneous pigs, inoculated with non-virulent tubercle bacilli and having chronic disease, were used. (See Table VI.) (A)

Result. There was apparently no influence on the temperature.

Test 3 *days later of the first four pigs* (Table VI., B) *with tuberculin.* Ass R, second serum, was given in separate places at the same time, subcutaneously.

TABLE VI. (B).

No.	Serum.		Tuberc.	Temperature.		
	Kind.	Dose.	Dose.	A. M.	P. M.	Variation.
1 . . .	Ass R 2	2.15 c.c.	0.015 c.c.	101°	105°	+4°
2 . . .	0	0	0.010	101	104	+3
3 . . .	0	0	0.015	102.4	104.6	+2.2
4 . . .	Ass R 2	2.70	0.0155	101.6	102.4	+1.8

Result. There was no effect in preventing a rise of temperature.

January 20, 1897. *Effects of serum on the temperature when given with small doses of tuberculin.* 10 pigs were inoculated with bacilli of moderate virulence. (1 died of enteritis.) (Table VII)

They were tested on the twenty-second day. The serum of ass R, second, and NaCl, 0.6 per cent. solution, were given in doses of 0.004 to 1 gramme of pig, diluted one-half; tuberculin in a dose of 0.0005 to 100 grammes of pig; they were given in separate places at the same time, subcutaneously.

Result. The controls seemed to have a greater rise of temperature, but the NaCl pigs seemed to have less rise than the serum animals, thus making a specific effect of the serum less likely.

January 20, 1897. *Serum of ass R, second bleeding, and of horse II.* 6 pigs of an average weight of 492.5 grammes were inoculated with a pure culture of tubercle bacilli.

TABLE VII.

No.	Weight.			Serum, etc.		Tuberc.	Temperature.		
	I/20,'97	II/11,'97	Gain or loss.	Kind.	Dose.	Dose.	12 M.	6 P.M.	Variation
1	665 gm.	605 gm.	—60gm.	Ass R 2	2.40 c.c.	0.003 c.c.	102.2°	102.2°	0
2	400	395	— 5	"	1.58	0.0019	103.6	105	+1.4°
3	390	400	+ 5	"	1.60	0.002	103.8	104.4	+0.6
4	420	415	— 5	"	1.66	0	102	102.6	+0.6
5	375	345	—30	NaCl	1.48	0 0017	102	101.6	—0.4
6	390	440	+50	"	1.76	.0.022	103	103.6	+0.6
7	445	450	+ 5	Control	0	.0.022	102.2	104.6	+2.4
8	610	610	0	0	0	0.003	102	103.6	+1.6
9	920	875	—45	0	0	0.0043	103	104.8	+1.8
10	.....	.....	.....	.....	.....	.....	.....	.....	.....

The test was on the twenty-~~fourth~~ ninth day. Serum was given in doses of 0.006 to 1 gramme of pig; tuberculin in doses of 0.0005 to 100 grammes of pig; they were given simultaneously, in separate places under the skin. (Table VIII A)

TABLE VIII. (A).

No.	Weight.			Serum. Ass R 2	Tuberc.	Temperature.		
	I/20,'97	II/18,'97	Gain or loss.	Dose.	Dose.	12.30 P.M.	5.45 P.M.	Variation.
1	475 gm.	450 gm.	—25gm.	2.70 c.c.	0.0022 c c.	102.8°	104.6°	+1.8°
2	555	545	—10	3.27	0.0032	102.2	Died soon after dose (shock)	
3	430	415	—15	2.49	0.0024	102.8	104.4	+1.6
4	480	400	—80	Control	0.0024	103.8	105.2	+1.4
5	500	535	+35	"	0.0020	103.2	104.4	+1.2
6	515	505	—10	"	0.003	102.6	102.8	+0.2

Result. The serum did not prevent the temperature rise due to tuberculin.

The serum of horse II was tested on the forty-second day. Serum was given in a dose of 0.004 to 1 gramme of pig; tuberculin in equal volume was given subcutaneously 10 minutes after the serum. (Table VIII B)

Result. There was no influence upon the effect of non-fatal, large doses of tuberculin.

January 5, 1897. Serums of horse I and horse II. 10 pigs were inoculated with a pure culture of tubercle bacilli of moderate virulence. (See Table IX.)

They were tested on the forty-ninth day. The serum and the tuberculin were given in separate places under the skin of the abdomen, simultaneously.

TABLE VIII. (B).

No.	Weight.			Serum.		Tuberc.	Temperature.		
	I/20,'97	III/4,'97	Gain or loss.	Kind.	Dose.	Dose.	10.30 A.M.	5.45 P.M.	Variation
4	480 gm.	380 gm.	—100gm.	Horse II	1.10 c.c.	0.076 c.c.	102°	104.4°	+2.4°
3	430	420	—10	"	1.68	0.084	102.2	105	+2.8
6	515	520	+5	"	2.08	0.104	102.4	99	—3.4
1	475	435	—40	Control	0	0.097	101.8	103	+1.2
5	500	555	+55	"	0	0.111	102	105	+3

Result. There was evidently no preventive effect on the temperature reaction.

March 27, 1897. *Effect of the serums of R and H on the local reaction after tuberculin injections into the eyes of rabbits* (see Part I.). Three weeks after discontinuance of treatment and 56 days after the inoculation of the eyes, the congestion of the acute stage in the disease was decreasing; some eyes had become caseous to a large extent. A small dose (0.010) of tuberculin was given to 5 of the animals (not mixed with serum); serum was given at the same time. 2 received 3 c.c. each of serum R; 2 received 3 c.c. each of serum H; 1 control received tuberculin only.

TABLE IX.

No.	Weight.			Serum.		Tuberc.	Temperature.		
	I/5,'97	II/23,'97	Gain or loss.	Kind.	Dose.	Dose.	12.30 PM	5.30 P.M.	Variation
1	440 gm.	280 gm.	—60gm.	Horse I	2.0 c.c.	0.002 c.c.	101.2°	105°	+3.8°
2	455	465	+10	"	1.5	"	102.4	105.4	+3
3	377	405	+28	"	1.5	"	103.2	104.4	+1.2
4	777	760	—17	Horse II	2.0	"	101.2	105.2	+4.0
5	360	410	+50	"	1.0	"	103	105.8	+2.8
6	760	630	—130	"	1.5	"	103.2	104.6	+1.4
7	407	465	+58	Control	0	"	102.2	104.8	+2.6
8	792	785	— 7	"	0	"	102.2	104.2	+2
9	490	470	—20	"	0	"	103	105.6	+2.6
10	685	690	+ 5	"	0	"	103	104.2	+1.2

Result. There was slight additional redness in all, with a temperature of 104.5°, an increase of 2° to 2.5° after six hours. This was not less with the serum rabbits.

April 1, 1897. The test was repeated with three controls; the dose of tuberculin was 0.020.

Result. There was slight increase of congestion and temperature in all, without perceptible difference between the controls and the serum animals. There was no influence in preventing local reaction.

RÉSUMÉ. The results of our four years' work in experiments upon four sheep, three asses, twelve fowls, eighteen rabbits, and four hundred and fifty guinea-pigs are to be found in the following summary :

1. A sheep was injected intravenously with killed thymus cultures. The result was so unsatisfactory that the serum-tests were not conclusive.

2. Chickens were inoculated intraperitoneally with mammalian tuberculosis. The serum revealed no germicidal or inhibitive action on the tubercle bacilli, nor favorable influence on the course of the disease in guinea-pigs.

3. A sheep was injected with tuberculin. The serum was wanting in germicidal, antitoxic, or curative effect so far as tested.

4. A sheep was inoculated intravenously with non-virulent cultures. Cachexia followed, and the serum was therefore not used.

5. An ass was inoculated as in (4.); it died from pulmonary embolus. The serum was not bactericidal to tubercle bacilli.

6. An ass was inoculated with virulent tubercle bacilli and treated with tuberculin. The serum showed no germicidal nor curative, but possibly some antitoxic effect.

7. An ass was inoculated with non-virulent tubercle bacilli and treated with various extracts of tubercle bacilli and dead bacilli. The serum showed no activity.

8. Rabbits were inoculated with non-virulent and virulent tubercle bacilli, and recovered. Their serum possibly conferred some protection in tuberculin poisoning, and possibly prolonged the lives of treated guinea-pigs.

With a full appreciation of the uncertainty of correct conclusions from tests of the serums other than our own product which were tried with tuberculin, we may state that only one indicated antitoxic power. This was obtained from a horse inoculated with non-virulent cultures.

That the apparent protection against fatal tuberculin poisoning occasionally seen was not necessarily due to the specific antitoxic power of the serums is made probable by the similar effects of physiological salt solution seen at times.

None of the serums appeared to prevent local or general reaction from small doses of tuberculin, nor to influence the temperature of tuberculous animals.

Disappointing as these results may seem, the writers feel that, in the light of recent contributions made by Ehrlich, Wasserman and Behring[10] to our knowledge of the mechanism of immunity and antitoxin production in the body, the outlook for an efficient tuberculosis antitoxin is by no means a hopeless one.

In conclusion, we would express our thanks to Drs. S. W. Hewetson, W. S. Nelson, and J. A. Wilder, all of whom have aided in the many arduous details of these experiments. Apl. 1, 1894.

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